Variation in Budburst Phenology of Douglas-fir Related to Western Spruce Budworm (Lepidoptera: Tortricidae) Fitness

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ABSTRACT Variation in budburst phenology among individual trees of interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) may influence their susceptibility to western spruce budworm (*Choristoneura occidentalis* Freeman) defoliation. We tested the hypothesis that phenological asynchrony between Douglas-fir and the western spruce budworm is a mechanism of resistance using clones derived from parent trees that showed resistance versus susceptibility to *C. occidentalis* defoliation in the field. Susceptible clones had earlier budburst phenology compared with resistant clones when they were grown in a common greenhouse environment, demonstrating a genetic basis for parallel phenological differences exhibited by the parent trees. We tested the importance of phenological asynchrony as a factor influencing fitness of *C. occidentalis* using two different greenhouse bioassay experiments. One experiment compared western spruce budworm performance on equivalent phenological stages of susceptible and resistant clones by matching larval feeding to the columnar (fourth) bud development stage of each clone. Larvae reared on resistant clones had greater realized fitness (i.e., number of F_1 offspring produced) than those reared on susceptible clones when the influence of variation in budburst phenology was minimized. In the other experiment, western spruce budworm larvae were placed on all trees on the same date when ≈50% of all terminal buds in the population were in the yellow (second) budburst stage. Larvae reared on susceptible clones had greater realized fitness than those reared on resistant clones when the influence of phenological asynchrony was expressed. Our results suggest that resistant phenotypes of Douglas-fir have negative effects on survival and reproduction of *C. occidentalis* under the natural conditions that insects and trees experience in the field. Genetic variation among trees in budburst phenology has an important influence on interactions between the western spruce budworm and Douglas-fir.

KEY WORDS *Choristoneura occidentalis*, *Pseudotsuga menziesii*, bioassay, budburst phenology, defoliation, resistance mechanisms.

Tree resistance plays an important role in the ecology of forest insects (Larsson 2002). Variation exists within many natural plant populations in traits that are likely to confer resistance to insects, including trees. Larsson (2002, p. 2) defined host plant resistance as "not primarily related to plant damage . . . but instead focuses on the negative effect a resistant plant has on the target herbivore." He considered "the interaction between the plant trait and the insect response to constitute the mechanism of resistance." Unfortunately, a poor mechanistic understanding of tree resistance has often precluded considering tree resistance in forest pest management strategies.

Clancy (2002) summarized the role of numerous potential mechanisms of resistance in trees to defoliators using western spruce budworm (*Choristoneura occidentalis* Freeman) and interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco)

as a model system. We chose to study this insect–plant system in detail because the western spruce budworm is the most important forest defoliator in western North America (Brookes et al. 1987), and Douglas-fir is a commercially important host tree species (Silen 1978, Hermann and Lavender 1990, Hardin et al. 2001).

Various mechanisms of resistance have been evaluated for this model system using a combination of laboratory diet bioassays (Clancy 1991b), field observations on pairs of mature Douglas-fir trees that are phenotypically resistant versus susceptible to damage from the western spruce budworm (Clancy et al. 1993, Clancy 2001), and greenhouse bioassays with grafted clones and half-sib seedling progeny derived from the resistant and susceptible trees (Chen et al. 2001a, 2002a, 2002b). Furthermore, we have documented protein allozyme differences between the resistant and susceptible parent trees (Chen et al. 2001b), suggesting that the phenotypic differences observed in resistance of these interior Douglas-firs to defoliation by the western spruce budworm are at least partly caused by genetic differences among trees.

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Three mechanisms appear to be important determinants of Douglas-fir resistance to the western spruce budworm: phenological asynchrony (Clancy et al. 1993, Chen et al. 2001a), vigor (i.e., growth rate) (Clancy et al. 1993; Chen et al. 2001a, 2002b), and low nutritive quality of foliage (Clancy 1991a, 1992, 2001, 2002; Clancy and King 1993; Clancy et al. 1993). However, five mechanisms have been excluded: compensatory photosynthesis (Chen et al. 2001a), toughness of needles (Burr and Clancy 1993, Clancy 2002), defensive compounds (i.e., monoterpenes) in foliage (Clancy et al. 1992, 1993; Clancy 1993, 2001; Chen et al. 2002a), induced defenses (i.e., induction of foliar monoterpenes) (Chen et al. 2002a), and western spruce budworm feeding and oviposition behavior (Palermo et al. 2003). The roles of induced susceptibility and ectomycorrhizal mutualists are currently under evaluation (Clancy 2002, Palermo 2002).

Phenological asynchrony between host trees and their insect herbivores is often an important mechanism of resistance for species that are early-season feeders on developing new buds and leaves of trees; early or late budburst can directly affect both the quantity and quality of suitable food available to herbivores at specific times (Kolb and Teulon 1991, Quiring 1992, Lawrence et al. 1997). If emergence of larvae is too early in relation to budburst of host trees, insects will be forced to disperse to find suitable food resources. Increased dispersal invariably leads to higher mortality from natural enemies or starvation. Alternatively, if larval emergence is too late, insects will be forced to feed on leaves or needles that are too mature to be an optimal food source; this can result in slower larval growth rates, smaller (and less fecund) adults, and increased larval mortality.

The western spruce budworm has a univoltine life cycle; larvae are early-season feeders on the expanding new buds and needles of host trees. Adult moths emerge in early July and typically mate within 24 h of eclosion; after mating, the females lay from two to eight clutches of eggs with each egg mass containing 25–40 eggs (Brookes et al. 1987). First instars hatch 7–10 d later, disperse from the egg mass, and spin hibernaculae in sheltered locations on host trees. Then they molt into second instars without feeding, and overwinter in their hibernaculae. Second instars emerge from their hibernaculae in early May of the following year, usually over a period of ≈10 d (Brookes et al. 1987); most of them passively disperse on silken threads, and upon landing on a suitable food source, begin feeding. Western spruce budworm larvae can damage all types and developmental stages of tissues (Frank and Jenkins 1986), but they prefer to feed on nutrient-rich tissues such as swollen buds, current-year needles, and pollen (Shepherd 1992, Dodds et al. 1996). Larvae can also mine less nutritious older needles as a survival strategy until swollen buds and new needles are available (Shepherd 1992). The larvae continue feeding as they molt through a total of six instars, and then they pupate on the foliage. The pupal stage lasts ≈10 d, after which adult moths emerge to repeat the cycle (Brookes et al. 1987).

Several lines of evidence suggest that differences in budburst phenology are an important mechanism of resistance in Douglas-fir trees that is under genetic control. First, field observations of budburst phenology of paired resistant and susceptible trees clearly showed that resistant trees from three different populations consistently had later budburst phenology than susceptible trees (Clancy et al. 1993, Clancy 2002). Second, Chen et al. (2001a) reported that clones of resistant trees had later budburst than clones of susceptible trees. They found that the resistant clones required ≈90–110 more degree-days to reach the same budburst stage as susceptible clones when the trees were grown in a common environment in greenhouses. Third, we have documented that open-pollinated seedlings from our resistant Douglas-fir phenotypes had consistently later budburst compared with those from our susceptible phenotypes (Z. Chen, unpublished data). Moreover, phenology of budburst is known to be a highly heritable trait in Douglas-fir (Silen 1978; Li and Adams 1993, 1994). These results all support a genetic basis for the differences we have observed in budburst phenology in the field.

The goal of this study was to test the hypothesis that phenological asynchrony between Douglas-fir and the western spruce budworm is a mechanism of resistance, *sensu* Larsson's (2002, p. 2) definition that "the interaction between the plant trait and the insect response . . . constitute the mechanism of resistance." We reared western spruce budworm larvae on resistant and susceptible clones in greenhouse bioassay experiments to establish how host tree phenotype affected *C. occidentalis* survival and reproduction (i.e., fitness) over one complete generation. Two different bioassays were used to assess the importance of budburst phenology as a factor determining host plant resistance. In one experiment, western spruce budworm feeding was matched to the bud flush of each individual plant to minimize the influence of variation among clones in budburst phenology. In the other experiment, *C. occidentalis* feeding was matched to bud flush of the whole population of plants so that the influence of phenological asynchrony was expressed, as often happens under natural conditions in the forest (Clancy et al. 1993).

Materials and Methods

Douglas-fir Trees and Clones. Our experimental plant material consisted of clones derived from mature Douglas-fir trees that differed in western spruce budworm defoliation under field conditions (Clancy et al. 1993). The mature Douglas-fir trees were from sites on the Pike National Forest near Deckers, CO (elevation = 2,573 m) and the Kaibab National Forest near Jacob Lake, AZ (elevation = 2,774 m). At the time the trees were identified (1988 and 1989) most trees at the sites had sustained moderate to severe western spruce budworm defoliation for at least several years, as determined from their growth form and general condition. We selected seven phenotypically resistant trees at the Pike National Forest site and five phenotypically

resistant trees at the Kaibab National Forest site by identifying trees with full crowns and little other evidence of western spruce budworm damage. These trees were visually distinct from other trees in the stand that were characterized as phenotypically susceptible based on their defoliated crowns. Each resistant tree was paired with a nearby (within 30 m) susceptible tree of similar size (height and diameter at breast height) and micro site (slope and aspect). In other words, the pairs of resistant and susceptible trees were "matched" as closely as possible to minimize any size-, age-, or micro site-related effects that could confound effects associated with different levels of herbivory.

We cloned each of the 24 mature trees by whip-grafting branches collected from the lower third of the crown onto 1-yr seedling rootstocks in 1991 and 1992. This is a common and widespread technique for reproducing mature tree characteristics in a smaller plant (Hartmann and Kester 1983, Zobel and Talbert 1984). Such cloning resulted in the fixation of the genotype and tissue developmental stage of mature trees but not tree environment. The cloned trees (i.e., ramets) were 6- to 7-yr-old in spring 1998 before the start of the western spruce budworm defoliation experiment; the average size (mean ± SE, $n = 288$) of the ramets was 93.5 ± 1.6 cm tall, 45.9 ± 0.7 cm in crown diameter, and 2.11 ± 0.03 cm diameter at the base of the stem (above the graft union). Throughout the experiments, all ramets were grown in 15.0-liter plastic pots (containing a mixture of screened peat moss and vermiculite) in the Rocky Mountain Research Station greenhouses in Flagstaff, AZ. They were watered and fertilized on a regular schedule to minimize physiological stress. The maximum photosynthetically active radiation in the greenhouses was ≈60% of ambient outdoor levels. Air temperature was monitored with thermographs, and relative humidity was monitored with computer-controlled sensors in the greenhouses.

Experimental Design. The experiment had a completely randomized block design composed of six blocks, each containing 48 clonally propagated trees (i.e., two treatments [western spruce budworm defoliation versus control] × two traits [resistant versus susceptible]/pair × 12 pairs). A block was a bench of 48 trees in one of two identical greenhouses; each greenhouse contained three blocks. In total, 288 cloned trees (i.e., ramets) were included in the experiments. However, 11 trees died before the experiment started, therefore, there were actually 4–6 replications of each treatment combination for each of the 12 pairs. A total of 136 ramets (66 resistant and 70 susceptible) were defoliated in the 1998 experiment, whereas 139 ramets (68 resistant and 71 susceptible) were defoliated in the 1999 experiment. Three of the defoliation treatment ramets were not defoliated in 1998 because their budburst was unusually late.

Budburst Phenology. The development of current-year buds and shoots was monitored on 8–20 (depending on the size of the ramet; 20 buds were used on most ramets) randomly selected, labeled terminal buds from the upper two-thirds of the crown on each undefoliated grafted tree in the 1998 experiment. In total, 1,284 buds and shoots were monitored on 68 resistant ramets, and 1,336 buds and shoots were monitored on 70 susceptible ramets. We recorded the developmental stage of each labeled bud/shoot weekly based on Shepherd's (1983) budburst scale from 19 March to 7 May 1998. The monitoring ended when ≈ 85% of the current-year shoots on each ramet were at or beyond the eighth (shoot growth) stage (Shepherd 1983). Budburst was plotted as a function of degree-days (DD) that were calculated using the following equation: $DD = \Sigma\ ((T_{max} + T_{min})/2 - T_b) \cdot t$ (Campbell and Norman 1998), where T_{max} and T_{min} are the daily high and low temperature in the greenhouse, respectively, T_b is the base metabolism temperature or threshold temperature (5.6°C for Douglas-fir; Wickman 1981), and t is time, or the number of days from the beginning to the end of the budburst monitoring period in 1998.

Western Spruce Budworm Bioassay. To test the role of budburst phenology as an influence on *C. occidentalis* performance, we conducted the defoliation experiment differently in 1998 and 1999. The larvae used in our study were from our laboratory cultures of diapausing and nondiapausing western spruce budworms, maintained in the Entomology Laboratory at the Rocky Mountain Research Station, Flagstaff, AZ. The nondiapausing colony was started in 1985 with egg masses obtained from the Canadian Forest Service's Forest Pest Management Institute in Sault Ste. Marie, Ontario (Clancy 1991c); Leyva et al. (1995) concluded that this colony has growth rates and feeding behavior similar to a wild population. The diapausing colony was established from wild late instar western spruce budworms collected in Arizona and Colorado in 1996–1998, so it has been in culture for fewer generations than the nondiapausing colony.

We had planned to use second instars in hibernaculae from our diapausing culture to defoliate the grafted trees in both years, but not enough larvae were available from this culture in 1998. Consequently, in the 1998 experiment, nondiapausing third and primarily fourth instars (one larva to five terminal buds) were used to defoliate each cloned tree when ≈50% of its buds were in the fourth (i.e., columnar) budburst development stage. This stage is highly suitable for *C. occidentalis* feeding (Shepherd 1983). Because larval feeding was purposely matched to the fourth budburst stage of each clone, the effect of genetic variation in budburst phenology among trees on western spruce budworm feeding was minimized (i.e., phenological asynchrony minimized). In the 1999 experiment, diapausing second instars in hibernaculae (one larva to four terminal buds) were introduced to the same clones that were defoliated in 1998. However, all larvae were placed on all the trees on the same date when ≈50% of all terminal buds in the population were in the second (i.e., yellow) budburst stage (Shepherd 1983). The average phenological stage of Douglas-fir trees in the field at the time of 50% emergence of budworm larvae will obviously vary among different

years and locations. However, data from Shepherd (1983) indicated that using the point when 50% of the terminal buds in the population were in the yellow stage is a reasonable approximation of the average synchronization between larval emergence and bud swelling under field conditions. This schedule of larval introduction allowed genetic differences in budburst phenology among trees to influence the developmental stage of buds available for western spruce budworm feeding (i.e., phenological asynchrony expressed), as can occur in Douglas-fir forests (Clancy et al. 1993).

Other experimental procedures were the same in both years. Both defoliated and nondefoliated tree clones were caged with nylon "No-See-um" netting bags (The Rain Shed Corp., Corvallis, OR) that allowed ≈80% of full light to penetrate to contain larvae and create similar growing conditions for all clones. The bags were not removed until 95% of the larvae pupated, which required ≈5 wk.

Most of the pupae were removed from defoliated tree clones within 24–48 h of pupation. Male and female pupae were separately weighed (to the nearest 0.1 mg), and sorted into trays based on clone genotype. The pupae were refrigerated at 10°C for up to 7 d until an adequate number of male and female pupae were collected from the same clone genotype for mating, which occurred in brown paper bags at room temperature (20°C).

When ≈10% of the moths had emerged in the mating bags, freshly clipped Douglas-fir foliage was added for adult oviposition substrate. Once the foliage was added, the moths were allowed to mate and oviposit for 7–10 d. Next, the foliage was removed and the number of next generation (noted as F_1) egg masses larger than 4 mm was counted. Then, mating bags were frozen to kill the adult moths, and the unemerged pupae (i.e., dead) in each bag were counted and sexed to measure percent survival through the pupal stage and the number of female moths that had emerged. The collected F_1 egg masses were placed in Petri dishes, sealed inside plastic bags, and incubated in the laboratory (20°C) for 7–10 d to determine if the egg masses were viable (i.e., if ≥50% of the eggs in the mass hatched).

Western Spruce Budworm Performance Measurements. *Choristoneura occidentalis* performance after feeding on the different tree genotypes was assessed in 1998 by (1) the percentage of third and fourth instars that survived to the pupal stage; (2) male and female pupal weight (female pupal weight is strongly correlated with potential fecundity, or the number of oocytes per female [$r^2 = 0.87$–$0.91, P < 0.001$; Wagner et al. 1987]); (3) the percentage of pupae that survived to the adult stage (based on the pupae that were placed in mating bags); (4) the percentage of F_1 egg masses that were viable (≥50% of the eggs in the mass hatched); (5) the average number of egg masses laid per female moth; and (6) the average number of F_1 eggs per egg mass (estimated from egg mass size (Clancy 1991b)). The latter two measures were used to estimate realized fecundity or the number of F_1 eggs laid per female moth (Clancy 1991b). Perfor-

mance in 1999 was assessed using measurements 2–6 only, because accurate calculation of larval survival to the pupal stage was not possible; this was because of the inadvertent survival of some residual F_1 larvae on some trees from the 1998 experiment.

Empirical data in 1998 and 1999 were further used to estimate realized fitness or *C. occidentalis* population growth over three generations on Douglas-fir trees that are resistant versus susceptible to defoliation by using following model modified from Clancy (1991b):

Number of F_1 larvae = [number of P_1 (i.e., first parent generation) larvae] (P_1 proportion [i.e., %] cohort survival to the adult stage) · (P_1 average realized fecundity per female) · (F_1 proportion egg masses with viable eggs).

The model estimated the number of first instars alive at the beginning of the F_1, F_2, and F_3 generations, assuming that the resistant and susceptible trees had equal populations at the beginning of the P_1 generation (i.e., we assumed that both populations started with a single P_1 larva). The numbers of F_2 and F_3 larvae were calculated by using the value calculated by the model for the number of F_1 or F_2 larvae alive at the beginning of that generation as the P_1 value. For the 1998 bioassay, survival to the adult stage was estimated as larval survival x pupal survival. Alternatively, for the 1999 bioassay, this value was based on survival through the pupal stage alone because the data on larval survival was unavailable as mentioned earlier.

We acknowledge that our estimations of western spruce budworm fitness were inflated in 1998 (because we did not have data on survival of second instars to the third- or fourth-instar stage) and in 1999 (because reliable data on survival of second instars to the pupal stage were unavailable). However, the lack of these data did not influence our comparisons between the fitness of *C. occidentalis* after feeding on clones of resistant and susceptible trees within each experimental year (i.e., the biases were consistent between the resistant and susceptible trees in each year).

Statistical Analysis. *Budburst Phenology.* We calculated the average budburst stage over the 8–20 buds we monitored per nondefoliated clone each week. Sampling dates were converted into Julian days to plot budburst as a function of degree-days. Curves were fit to the distribution of budburst stage as a function of degree-days using SigmaPlot© (SPSS Inc. 2000).

Western Spruce Budworm Performance. Data from the 1998 and 1999 experiments were analyzed separately. The average fresh weight of all the male or female pupae collected from each defoliated tree (5–91 male or female pupae per tree) was calculated to avoid pseudoreplication (i.e., the tree was the experimental unit). The data were unbalanced because some of the 136 (1998) or 139 (1999) defoliated clones did not produce any male or female pupae. The analysis of variance (ANOVA) for pupal weights was based on a general linear model (y = block + pair +
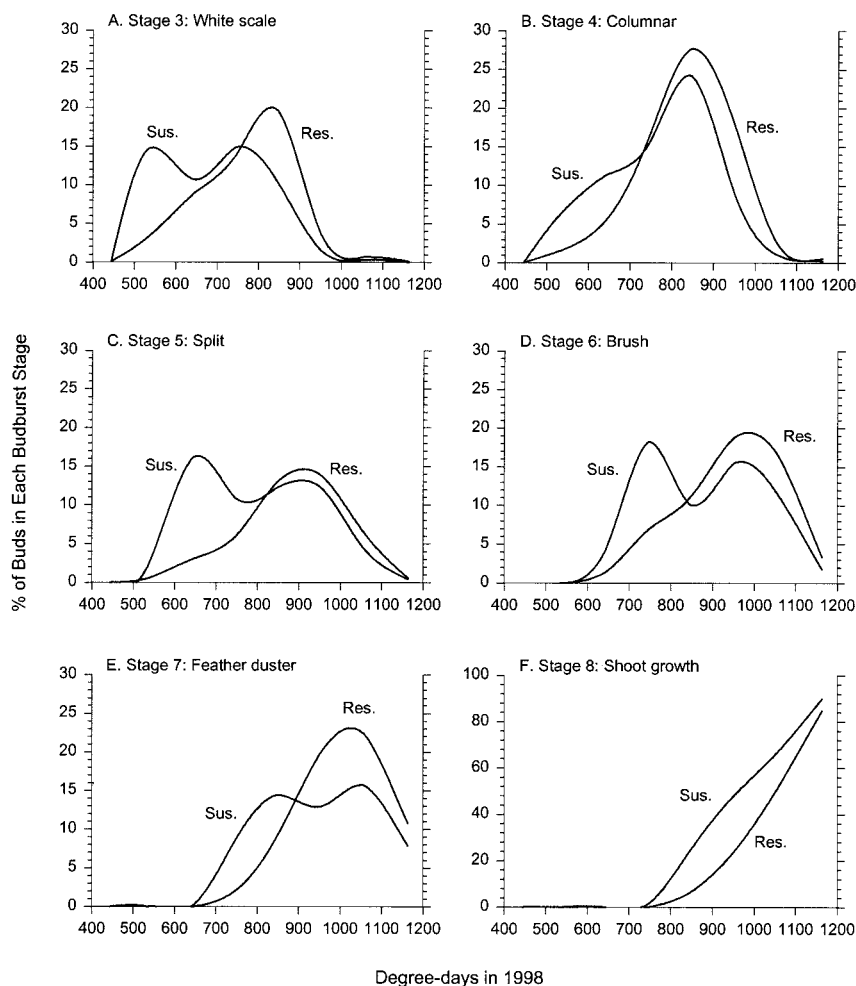
Fig. 1. The percentage of current-year buds in phenological stages 3–8 (A–F) plotted against degree-days in 1998 for clones of Douglas-fir trees that were resistant (Res.) versus susceptible (Sus.) to western spruce budworm defoliation. Phenological scores and terminology are according to Shepherd (1983).

trait + gender [♀ versus ♂] + [pair × trait] + [pair × gender] + [trait × gender], where y is the average male or female pupal weight, $n = 209$ in 1998 and $n = 204$ in 1999). This ANOVA had normally distributed residuals. All statistical analyses were performed with SAS (SAS Institute 1990).

The data on various western spruce budworm performance measures were pooled over the four to six clones derived from each of the 12 pairs of parent trees that showed resistance versus susceptibility to defoliation. This allowed us to obtain larger sample sizes for calculating percentage survival through various life stages, and for measuring average fecundity of the female moths. Paired t-tests were used to analyze each response variable (i.e., to compare responses between the resistant and susceptible clones, $n = 12$ pairs). Finally, we pooled categorical data on survival through various life stages over all the resistant or susceptible clones, and used χ^2 tests to determine if survival varied between the resistant versus susceptible phenotypes.

Results

Budburst Phenology. Some interesting differences in the patterns of budburst between resistant and susceptible clones emerged when we plotted the distribution of buds in various phenological stages against DD (Fig. 1). The resistant clones had unimodal (i.e., approximately normal) distributions of bud stages 3–7 over DD (Fig. 1A–E). Alternatively, the susceptible clones had distinct bimodal distributions for bud stages 3, 5, 6, and 7 (Fig. 1A and C, D, E), and a skewed distribution for bud stage 4 (Fig. 1B). Although all of these stages are suitable for western spruce budworm feeding, the swollen buds (i.e., stage 3–4) are perhaps the most nutrient-rich tissues for them (Shepherd 1992). This implies that the susceptible trees had broader phenological "windows" when a given percentage of their buds and shoots were in stages of development suitable for larval feeding. For example, at least 10% of the total buds were in stage 3 of development for ≈368 DD (i.e., the phenological "win-

Table 1. Analysis of variance results for fresh pupal weights of western spruce budworms feeding on clones of resistant versus susceptible Douglas fir trees in the 1998 and 1999 greenhouse bioassays

Source of variation	df	1998 Phenological asynchrony minimized Pupal weight ($n = 209$)		1999 Phenological asynchrony expressed Pupal weight ($n = 204$)	
		F	P	F	P
Block	5	6.23	<0.001	3.53	0.005
Pair	11	2.44	0.008	<1	0.666
Trait (resistant vs. susceptible)	1	3.05	0.083	<1	0.836
Gender (M or F)	1	63.7	<0.001	465	<0.001
Pair × Trait	11	1.09	0.376	<1	0.507
Pair × Gender	11	<1	0.943	1.41	0.173
Trait × Gender	1	<1	0.493	<1	0.697
Error					
1998	167				
1999	162				

See text for details about the bioassays, *ANOVA* model, and sample sizes.

dow" was 368 DD long) for the susceptible clones, whereas the comparable "window" for the resistant clones was only ≈241 DD (Fig. 1A). Similar "windows" for the susceptible versus resistant clones were 340 versus 304 DD for bud stage 4 (Fig. 1B), 396 versus 212 DD for bud stage 5 (Fig. 1C), 396 versus 283 DD for bud stage 6 (Fig. 1D), and 382 versus 312 DD for bud stage 7 (Fig. 1E).

Effects of Variation in Budburst Phenology on Western Spruce Budworm Performance. *Pupal Weight.* There were no detectable tree trait × insect gender interactions in either the 1998 or 1999 bioassays (Table 1), indicating that male and female fresh pupal weights were affected in a similar manner by the resistant versus susceptible clones in each year. Thus, we combined weights of both male and female pupae in a single ANOVA rather than analyze males and females separately.

In the 1998 experiment, when the influence of phenological asynchrony was minimized, fresh pupal weight of western spruce budworms reared on the resistant clones was slightly but not significantly higher on average (96.3 ± 1.74 mg [mean ± SE]) compared with those that fed on the susceptible clones (92.0 ± 1.80 mg) (Table 1). This result implies that the resistant clones provided a marginally better food source for the larvae than the susceptible clones did, when the third or fourth through sixth instar feeding period was matched to the bud flush of each individual plant. Pupal weight varied significantly among the 12 pairs of clones, and between males and females. Female pupae weighed more (104 ± 1.76 mg) on average than males (84.3 ± 1.76 mg).

In the 1999 bioassay, when the influence of phenological asynchrony was expressed, fresh pupal weight was equivalent for insects that fed on the resistant versus susceptible clones (Table 1). There were also no detectable differences among the 12 pairs of clones. Female pupae in 1999 again weighed significantly more (147 ± 1.57 mg) than male pupae (99.4 ± 1.62 mg).

Survival Rate. When the western spruce budworm's larval feeding period was carefully matched to the bud phenology of each individual plant in 1998,

larval survival was significantly higher for insects feeding on the resistant clones compared with those feeding on the susceptible clones (Table 2A). The 1999 data on larval survival is not presented because it was unavailable.

Percentage survival from the pupal to adult (moth) stage was equivalent on the resistant and susceptible clones in both the 1998 and 1999 bioassays (Table 2B). Pupal survival was very high, ranging from ≈93–95%. Cohort survival rate from the third or fourth instar to the adult moth stage in 1998 was 64.1 ± 2.80% on resistant clones and 51.2 ± 3.67% on susceptible clones (paired $t_{11} = 2.76$; $P = 0.019$). It was not calculated in 1999.

Western spruce budworms that fed on the susceptible clones consistently tended to have a higher percentage of viable F_1 egg masses than those feeding on the resistant clones, although the differences were not significant (Table 2C). When the data were pooled over 1998 and 1999, insects feeding on the susceptible clones produced a marginally (but not significantly) greater percentage of viable F_1 egg masses (60.4 ± 2.21%) compared with those reared on the resistant clones (54.0 ± 3.83%) ($F_{1, 22} = 3.03$; $P = 0.096$).

Realized Fecundity and Fitness. Realized fecundity was similar between insects reared on resistant and susceptible clones in our 1998 and 1999 bioassays (Table 2D). There were also no significant differences in realized fitness when larvae were reared on the resistant versus susceptible clones (Table 2E), primarily because of the large variation among the 12 parent tree pairs. Nonetheless, there were interesting trends in realized fitness. *Choristoneura occidentalis* fitness was ≈25% higher on average on the resistant trees in the 1998 bioassay, when the larval feeding period was matched to the budburst phenology of each individual plant. However, this pattern was reversed in the 1999 bioassay; western spruce budworm fitness was ≈14% higher on average on the susceptible trees when the larval feeding period was matched to budburst phenology of the whole population of plants.

These trends were substantiated when we pooled all the data for the resistant or susceptible clones for each year (Table 3) and then calculated realized fit-

Table 2. Bioassay data on survival through the larval, pupal, and egg stages, plus realized fecundity and fitness per female moth, for western spruce budworms feeding on resistant versus susceptible Douglas fir clones in the 1998 and 1999 greenhouse experiments

Variable	Year	Phenological asynchrony	Trait	Measure (mean ± SE)	Paired t-test[a]	
					t	P
A. Survival from the larval to pupal stage						
	1998	Minimized	Resistant	67.50 ± 3.13%	2.51	0.029
			Susceptible	54.23 ± 3.97%		
	1999	Expressed	Resistant[b]	–	–	–
			Susceptible[b]	–		
B. Survival from the pupal to adult (moth) stage						
	1998	Minimized	Resistant	95.22 ± 0.92%	0.42	0.686
			Susceptible	94.54 ± 1.23%		
	1999	Expressed	Resistant	95.00 ± 1.59%	0.66	0.522
			Susceptible	93.38 ± 1.60%		
C. Survival through the F_1 egg stage[c]						
	1998	Minimized	Resistant	54.55 ± 4.10%	0.92	0.381
			Susceptible	59.08 ± 2.36%		
	1999	Expressed	Resistant	53.51 ± 6.61%	1.26	0.235
			Susceptible	61.68 ± 3.82%		
D. No. F_1 eggs laid per female moth (i.e., realized fecundity)						
	1998	Minimized	Resistant	82.94 ± 6.21 eggs	0.24	0.818
			Susceptible	80.98 ± 6.56 eggs		
	1999	Expressed	Resistant	114.19 ± 12.06 eggs	0.15	0.882
			Susceptible	116.56 ± 5.79 eggs		
E. No. F_1 larvae produced per female moth (i.e., realized fitness)						
	1998	Minimized	Resistant	29.57 ± 3.67 larvae	1.33	0.211
			Susceptible	23.62 ± 2.00 larvae		
	1999	Expressed	Resistant	59.04 ± 12.42 larvae	0.63	0.545
			Susceptible	67.40 ± 5.62 larvae		

See text for details about the bioassays.

[a] Results from paired t-tests comparing survival, fecundity, or fitness between the resistant and susceptible clones, $n = 12$ pairs.

[b] The 1999 bioassay data on larval survival was unavailable due to the survival of some residual F_1 larvae on some trees from the 1998 experiment.

[c] Percentage of F_1 egg masses with ≥50% of the eggs in the mass hatched.

ness for three consecutive generations (Fig. 2). It is clear that western spruce budworm populations would grow more rapidly on the resistant than on the susceptible Douglas-fir trees under the artificial conditions (i.e., phenological asynchrony minimized) we created in the 1998 bioassay (Fig. 2A–C). By the F_3 generation, population growth was 67% greater on the resistant trees (Fig. 2C). Alternatively, under the more natural conditions we simulated in the 1999 bioassay (i.e., phenological asynchrony expressed), population growth was greater on the susceptible than on the resistant Douglas-fir trees (Fig. 2D–F). In this case, western spruce budworm population growth was 37% greater on the susceptible trees by the F_3 generation (Fig. 2 F).

Discussion

Are There Interactions between Western Spruce Budworm and Tree Genotypes?

Based on our experimental results, we conclude that genetic variation among trees in budburst phenology is an important mechanism of resistance influencing interactions between the western spruce budworm and its Douglas-fir host trees. However, an alternative explanation is that the different results from the two experiments might have been caused by the use of insects from our nondiapausing colony in the 1998 bioassay versus insects from our diapausing colony in the 1999 bioassay. This interpretation would require assuming that the nondiapausing strain of *C. occidentalis* preferred (i.e., was adapted to have better survival and reproduction on) the resistant tree genotypes, whereas the diapausing strain preferred the susceptible tree genotypes. Conversely, we made the more parsimonious assumption that the resistant and susceptible Douglas-fir genotypes would have similar effects on the performance of the two western spruce budworm strains because we had no *a priori* reason to believe otherwise. We acknowledge that ideally we should have used the diapausing strain of insects for both experiments, and that was our original plan, but we did not have enough insects available from our diapausing culture in 1998. Nonetheless, we have no reason to believe that our experimental methodology invalidates our conclusions.

The Importance of Phenological Synchrony in Determining Population Growth and Defoliation Differences

The emergence of early-season insect defoliators is typically closely synchronized with budburst phenology of their host trees. If this synchrony is disrupted,

Table 3. Data used to predict realized fitness of western spruce budworms feeding on clones of resistant versus susceptible Douglas fir trees in the 1998 and 1999 greenhouse bioassays, including χ^2 test results for the effect of tree trait on western spruce budworm survival through the larval, pupal, and egg stage

Variable	Year	Phenological asynchrony	Trait	Measure	χ^2	P	Sample sizes
A. Survival from the larval to pupal stage[a]	1998	Minimized	Resistant	68.4%	74.6	<0.001	2,022 P_1 3rd–4th instars
			Susceptible	54.8%			1,835 P_1 3rd–4th instars
B. Survival from the pupal to adult (moth) stage	1998	Minimized	Resistant	94.8%	0.185	0.667	901 P_1 pupae
			Susceptible	94.3%			629 P_1 pupae
	1999	Expressed	Resistant	95.0%	1.03	0.310	320 P_1 pupae
			Susceptible	93.3%			552 P_1 pupae
C. Viability of F_1 egg masses	1998	Minimized	Resistant	54.8%	2.42	0.119	997 F_1 egg masses
			Susceptible	58.6%			700 F_1 egg masses
	1999	Expressed	Resistant	58.5%	1.95	0.163	248 F_1 egg masses[b]
			Susceptible	64.0%			394 F_1 egg masses[b]
D. No. F_1 eggs laid per female moth (i.e., realized fecundity)	1998	Minimized	Resistant	79.9 eggs	–[c]	–	432 P_1 female moths laid 997 F_1 egg masses with an average of 34.6 eggs each
			Susceptible	78.9 eggs			306 P_1 female moths laid 700 F_1 egg masses with an average of 34.5 eggs each
	1999	Expressed	Resistant	108.6 eggs	–	–	148 P_1 female moths laid 402 F_1 egg masses with an average of 40.0 eggs each
			Susceptible	112.2 eggs			270 P_1 female moths laid 766 F_1 egg masses with an average of 39.5 eggs each

See text for details about the bioassays.

[a] The 1999 bioassay data on larval survival was not used in the model because we determined it was invalid due to residual F_1 larvae on the plants from the 1998 defoliation treatments.

[b] Only a subsample of the F_1 egg masses collected from the mating bags was incubated to determine viability in the 1999 bioassay.

[c] The data on realized fecundity were calculated from data on the total number of F_1 egg masses laid by female moths in the mating bags, and the average number of eggs in each egg mass (estimated from the average egg mass area), so it was not appropriate to analyze it with a χ^2 test.

survival and reproduction of the herbivores can be significantly reduced (Quiring 1994). The relationship between susceptibility of host trees to early-season defoliators and budburst phenology has been widely investigated on both broad-leaved trees (Kolb and Teulon 1991; Hunter 1992; Marino and Cornell 1993; Dongen et al. 1996; Teulon et al. 1998, 1999) and conifers (Eidt and Cameron 1971, Shepherd 1992, Lawrence et al. 1997, Alfaro et al. 2000, Ostaff and Quiring 2000). For example, Lawrence et al. (1997) defined the phenological "window" for the (eastern) spruce budworm (*C. fumiferana* [Clemens]) feeding on white spruce (*Picea glauca* [Moench] Voss). They concluded that survival and reproduction of *C. fumiferana* were negatively affected by mismatched phenology between the insect's feeding period and bud development of the host trees. Our results with the western spruce budworm and Douglas-fir provide additional empirical evidence that synchrony between larval emergence and budburst phenology is critical for early-season feeders such as various *Choristoneura* species.

The differences in budburst phenology between the resistant and susceptible clones also influenced the amount of defoliation the trees sustained in the greenhouse bioassays (Chen et al. 2001a). In the 1998 experiment, when third and fourth instars were placed on each tree when the buds were highly suitable for feeding, the resistant clones were defoliated ≈23% more than susceptible clones. This pattern was re-versed in the 1999 bioassay, when diapausing second instars were placed on all trees on the same date regardless of budburst development. In this case, resistant clones were defoliated ≈25% less than susceptible clones. The defoliation data are also in agreement with data on *C. occidentalis* fitness; western spruce budworms had ≈19% greater realized fitness on resistant clones in 1998 when phenological asynchrony was minimized (Fig. 2A). Conversely, realized fitness was ≈11% greater on the susceptible clones in 1999, when phenological asynchrony was expressed (Fig. 2D).

Our experiments demonstrated that small phenological differences among individual Douglas-fir trees can have large effects on insect population growth (Fig. 2) and defoliation (Chen et al. 2001a). The 11% higher rate of western spruce budworm population growth we estimated for the susceptible clones under the more natural situation when phenological asynchrony is expressed can lead to dramatic differences in insect populations on resistant versus susceptible trees when multiplied over many insect generations. *Choristoneura occidentalis* populations would be 37% greater on susceptible compared with resistant trees after three generations (Fig. 2F), and this difference would compound to ≈183% more insects on susceptible trees after 10 generations.

These results also emphasize the importance of conducting bioassays over one complete generation to gain a clear understanding of how mechanisms of host
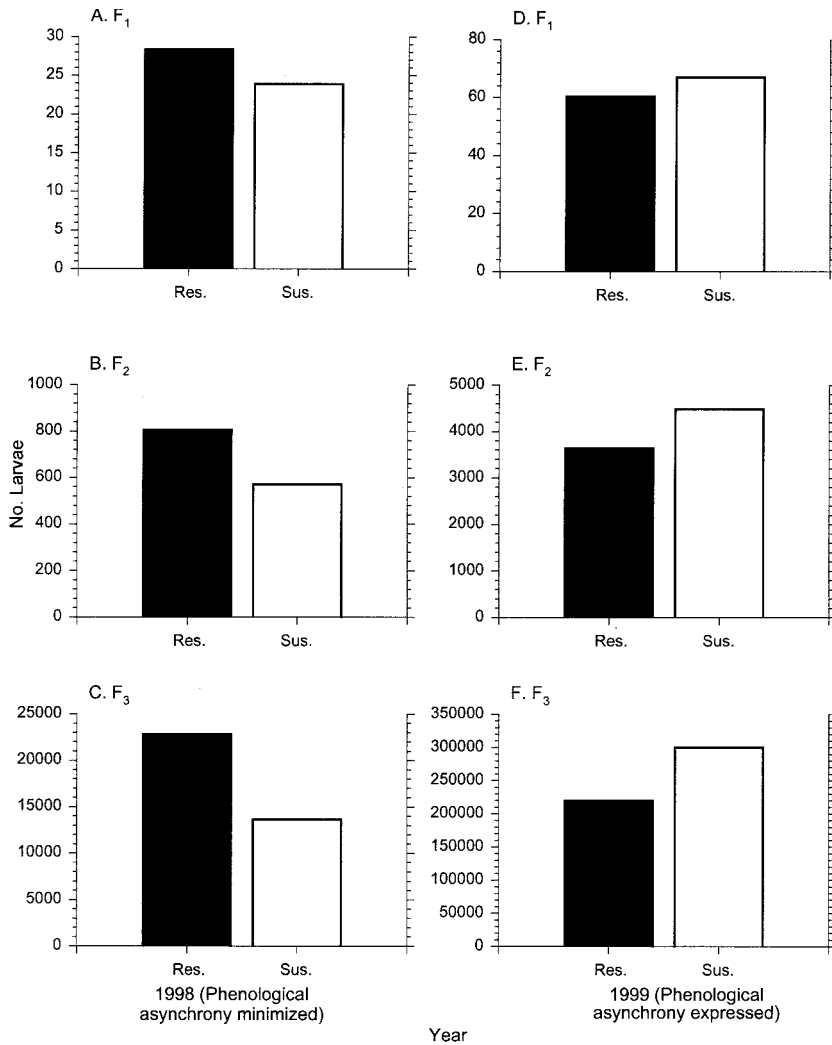
Fig. 2. Realized fitness, or the number of larvae alive at the beginning of the F_1 (A, D), F_2 (B, E), or F_3 (C, F) generations for western spruce budworms feeding on clones of resistant (■) versus susceptible (□) Douglas-fir trees in the 1998 (A–C) and 1999 (D–F) greenhouse bioassays. See text for details about the bioassays and equations used to calculate fitness. Realized fitness was calculated by pooling the data across all the resistant or susceptible clones for each year, so these are composite measures with no replication, accounting for the lack of error bars.

plant resistance to insect herbivores actually function to affect the population dynamics of the insects. For example, we would have come to very different conclusions about how budburst phenology affected western spruce budworm performance if we had focused on a single measure, such as realized fecundity (Table 2D). However, when we combined the empirical data on survival through various life stages (including the egg stage) with data on realized fecundity to predict fitness (i.e., population growth) over multiple generations, a very different picture emerged (Fig. 2).

What is the Cost of Resistance?

Phenology of budbreak is a critical physiological characteristic of all trees that grow in climates requir-

ing annual cycles of growth and dormancy. Trees that flush earlier in the spring gain the advantage of a longer growing season, suggesting that an obvious cost associated with the later budbreak phenology of the resistant Douglas-firs in our study is a shorter growing season compared with the susceptible trees that break bud earlier. Moreover, heavy selection pressure against the susceptible trees by western spruce budworm defoliation is sporadic in time and space. Budworm population outbreaks occur about every 20–33 yr on average, and they last ≈11 yr (Swetnam and Lynch 1993). Damage from the western spruce budworm is only one of many selective pressures that determine intraspecific variation in the reproductive success of interior Douglas-fir trees. The earlier budbreak phenology that is a disadvantage when *C. occidentalis* populations are high may confer important

selective advantages to individual trees during the periods between population outbreaks.

Acknowledgments

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